

INACTIVATION OF *SHEWANELLA PUTREFACIENS* BY GAMMA IRRADIATION OF RED MEAT AND POULTRY

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ABSTRACT

The resistance of Shewanella putrefaciens ATCC 8071, 8072, and 8073 to gamma radiation was determined in the presence and absence of air on mechanically deboned chicken meat (MDCM). The presence or absence of air (oxygen) did not significantly influence resistance to gamma radiation at 5C, and it was very sensitive with a D₁₀ value of 0.11 ± 0.002 kilogray (kGy) on MDCM. A high percentage of cells surviving irradiation were shown by impedance measurements to have suffered injury. The bacteria were significantly more resistant to gamma radiation at temperatures below the freezing point. At a dose of 0.8 kGy lowering the temperature of irradiation by 10 degrees increased the survival of this food spoilage organism by a 1.66 log₁₀. The type of meat (hamburger, ground beef round, ground pork, and ground turkey breast) did not significantly alter resistance of S. putrefaciens to gamma radiation under identical conditions (D₁₀ value = 0.18 ± 0.01 kGy). The minimum radiation dose currently approved for poultry in the USA, 1.5 kGy, should eliminate S. putrefaciens from meats.

INTRODUCTION

Food irradiation has been demonstrated to be a valuable treatment for the control of vegetative Gram-negative pathogenic bacteria on meat and poultry. Some D₁₀ values at 5C reported for *Campylobacter jejuni* (Clavero *et al.* 1994), *Escherichia coli* O157:H7 (Thayer and Boyd 1993), and *Salmonella* (Thayer *et al.* 1990) are 0.20, 0.28, and 0.39-0.77 kGy, respectively. These D₁₀ values indicate that significant reductions in the populations of these pathogens will be obtained at doses between 1.5 to 3.0 kGy that are approved for the irradiation

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of poultry in the U.S.A. (FSIS 1992). The effect of such radiation doses on nonpathogenic food spoilage bacteria are not as well known.

Shewanella putrefaciens (previously *Pseudomonas putrefaciens*, and *Alteromonas putrefaciens*) is described by MacDonell and Colwell (1985) as straight or curved rods, Gram negative, nonpigmented, motile by polar flagella, chemo-organotrophic, oxidase positive, and G+C mole % of 44-47. It is a common psychrotrophic spoilage organism with pink colonies; it degrades cysteine, and under anaerobic conditions H₂S is released with obvious effects on both meat color and odor (Gill and Newton 1979; Ringo *et al.* 1984). It is associated with spoilage of red meats, poultry, and fish (Gallo *et al.* 1988; Gill and Tan 1980; Gill 1983; Jørgensen and Huss 1989; Mast and Stephens 1973; McMeekin and Patterson 1975; Parker and Levin 1983; Seelye and Yearbury 1979; Viehweg *et al.* 1989). No previous studies of the radiation resistance of *S. putrefaciens* were found.

MATERIALS AND METHODS

Bacterial Cultures

S. putrefaciens ATCC 8071, 8072, and 8073 were obtained from the American Type Culture Collection, Rockville, MD, and maintained in tryptic soy broth (TSB, Difco) and on tryptic soy agar (TSA, Difco). Cultures were transferred to 10 mL of TSB and incubated for 24 h at 26C. One mL of this culture was used as the inoculum for 100 mL of TSB in a 500 mL baffled shake flask. The culture was incubated aerobically at 26C for 18 h with shaking at 150 rpm prior to use. After 18 h incubation, sufficient early stationary phase cells were harvested by centrifugation ($1,725 \times G$ at 5C for 30 min) and resuspended in one-tenth volume of Butterfield's phosphate (Pertel and Kazanas 1984) to provide an inoculum resulting in ca. 10^9 CFU/g (colony forming units per gram of meat) when it was mixed with the meat. The amount of culture needed was estimated from preliminary experiments and plate counts.

Meat Preparation

Mechanically deboned chicken (MDCM) consisting of approximately 90% rib and 10% back meat was obtained from a commercial manufacturer of poultry frankfurters. Fresh lean ground round of beef, ground beef (hamburger), lean ground pork loin, and ground turkey were obtained from local grocery stores. Each lot of meat was mixed well and vacuum packaged in 100 g amounts in Number 400 polyethylene Stomacher® bags (Tekmar Co., Cincinnati, OH) that were themselves vacuum sealed within a barrier bag (Freshstuff®, American National Can Company, Des Moines, Iowa). After freezing the meat was

sterilized with a gamma radiation dose of 42 kGy at -30C. The proximate analyses of these meats were published previously (Thayer and Boyd 1994).

Radiation Source and Irradiation Techniques

The self-contained, 134,000 Ci (4.95 PBq), gamma radiation source of ^{137}Cs had a dose rate of approximately 0.11 kGy/min. Dose rate was established using National Physical Laboratory (Middlesex, U.K.) dosimeters and corrected mathematically every week. Variations in absorbed dose were minimized by using thin samples, approximately 2 mm, placed within a uniform portion of the radiation field. Samples were maintained within $\pm 1.0\text{C}$ of the desired temperature during irradiation by injecting the gas phase from liquid nitrogen into the chamber and with continuous monitoring during irradiation.

Effect of Air Versus Vacuum on Gamma Radiation D-Values

Sterile MDCM was thawed rapidly by submersion of the package in a 50C water bath and then mixed with ca. $9.75 \pm 0.11 \log_{10}$ CFU/g of stationary-phase *S. putrefaciens* (prepared from equal volumes of ATCC 8071, 8072, and 8073) and suspended in Butterfield's phosphate by stomaching for 90 s using a Stomacher® 400 (Tekmar Co., Cincinnati, OH). Samples of 5.0 ± 0.05 g of the inoculated MDCM were transferred to sterile Number 400 polyethylene Stomacher® bags and spread uniformly over an area of about 10×10 cm within each bag. The bags were either vacuum (ca. -990 millibar) packaged and heat sealed or heat sealed with air in the bag. Each sealed bag was then vacuum packaged within a Freshstuff® bag to prevent oxygen absorption by the vacuum packed samples and to provide additional microbiological security for both sets of samples during irradiation. Samples were exposed to gamma radiation doses of 0 to 0.7 kGy in increments of 0.10 kGy at an irradiation temperature of $5.0 \pm 1.0\text{C}$. Following irradiation the samples containing air were immediately opened aseptically and flushed with air by opening and closing the bag to expel any residual ozone to prevent additional toxicity from ozone rather than from the radiation. Since the amount of ozone and its availability to the bacteria would be highly variable we felt that it should be eliminated so that the D-value obtained would be due to initial radiation effects and not due to residual amounts of ozone. Further, oxygen permeable packaging is required, at this time, for the irradiation of poultry, thus any residual ozone would diffuse from such packages. This study was repeated twice.

Microbiological Analysis

Samples were assayed for CFU by standard pour-plate procedures using TSA with serial dilutions in sterile Butterfield's phosphate. Petri plates were

incubated for 96 h at 26C. Colony forming units (CFU) were counted on three petri plates that had 30 to 300 colonies with a New Brunswick Scientific Biotran II automated colony counter.

Impedance Analysis of Cell Injury

Impedance analyses were performed using the bioMérieux Vitek Bactometer. The number of surviving CFU for each sample was determined as described above by standard pour plate analysis. Samples of 1.0 mL for assay of detection time were withdrawn from a 10^{-2} dilution used for plate counting and mixed with 9 mL of Wilkins-Chalgren Anaerobe broth (Oxoid, Ltd. Basingstoke, Hampshire, England). Aliquots of 2.0 mL were placed in two assay wells of the Bactometer plate to determine impedance detection times at 26C. Standard impedance detection time response curves providing estimates of the expected detection time for a given population of uninjured cells were obtained by analysis of serial dilutions of the unirradiated control samples in each study. The difference between the expected detection time and the observed detection time represents the injured population (Mackey and Derrick 1984; Thayer and Boyd 1994; Thayer *et al.* 1995a; Thayer *et al.* 1995c). The \log_{10} CFU/g for the average detection times were back calculated from the respective regression equations for irradiated and nonirradiated populations using the observed detection times. These values were converted back to the nonlogarithmic form, and the differences between the expected and actual populations were used as an estimate of the injured population. Because the two equations did not have exactly the same point of origin, these values were adjusted so as to be identical.

Effect of Temperature During Irradiation on Survival

Two 100-g samples of sterile MDCM were inoculated with ca. $9.56 \log_{10}$ CFU/g of a mixture of *S. putrefaciens* ATCC 8072 and 8073 and stomached for 90 s. (ATCC 8071 was not used in this study because it failed to grow). Aliquots of 5.0 ± 0.05 g of the inoculated MDCM were transferred aseptically to sterile Stomacher® bags and spread over an area of ca. 10×10 cm within the bags and heat sealed *in vacuo*. The inoculated meat samples (2 replicates) each received a dose of 0.50 kGy at irradiation temperatures of -20 to +20C at intervals of 5C. The surviving CFU/g in each sample were determined by standard pour plate analysis.

The results of the experiment described above indicated a need for a study of the effect of irradiation dose and temperature on the survival of *S. putrefaciens*. Sterile ground turkey breast was inoculated with 8.973 CFU/g of a mixture of *S. putrefaciens* ATCC 8071, 8072, and 8073. Aliquots of 5.0 g of the inoculated meat were vacuum packed in Stomacher® bags, as described above. A modified response surface design was used with single 5.0 ± 0.05 g samples

at 0, 0.4, and 0.8 kGy at -15C; 0, 0.2, and 0.6 kGy at -10C; 0 and 0.8 kGy at -5C; 0, 0.2, and 0.6 kGy at 0C; and 0, 0.4, and 0.8 kGy at 5C. Five samples at the center point of the design were irradiated to 0.4 kGy at -5C.

Comparison of Radiation Resistance on Red Meats and Turkey

Sterile hamburger, ground beef round, ground pork, and ground turkey were inoculated with $\log_{10} 8.51 \pm 0.24$ CFU/g of a mixture of *S. putrefaciens* ATCC 8071, 8072, and 8073. All four meats were used in each of the 3 independent replicate studies. Inocula were mixed with the meat and aliquots prepared as described above. The samples were vacuum packed and irradiated at a temperature of 5.0 ± 1.0 C.

Statistical Analysis

Radiation D-values were determined by least squares analysis of the means of the surviving populations, excluding the 0 kGy data to avoid possible shoulder effects, using the GLM procedure of the Statistical Analysis System (SAS) statistical package (Freund *et al.* 1986; Statistical Analysis System Institute, Inc. 1987). Regression techniques were used to fit second order response surface models (Draper and Smith 1981). Regressions were tested for differences by analysis of covariance and individual means by ANOVA. Unless stated otherwise, significance is expressed at the 0.05% level.

RESULTS

The radiation D-values for *S. putrefaciens* in MDCM were 0.11 ± 0.002 and 0.12 ± 0.002 in air and vacuum, respectively. Since these values were not significantly different the results were pooled, and the following D-value was calculated: 0.11 ± 0.002 kGy. Cells surviving radiation doses of 0.5, 0.6, and 0.7 kGy were estimated to have sustained ca. 45, 64, and 70% injury, respectively. We were unable to estimate the injury to the cells at doses of less than 0.5 kGy.

The temperature during irradiation to an absorbed dose of 0.50 kGy significantly affected the survival of a mixture of *S. putrefaciens* ATCC 8072 and 8073 in vacuum packed MDCM (Fig. 1). Survival at -20C was significantly greater than at -5, 0, 5, 10, 15, and 20C. Survival at -15C and -10C were significantly greater than at -5, 0, 5, 10, 15, and 20C. The results are interpreted as indicating that the survival of irradiated *S. putrefaciens* will be inversely proportional to the temperature of irradiation.

The temperature dependence indicated by the study in Fig. 1 led us to conduct a more detailed response surface analysis of the effect of temperature

with respect to dose. The response is described by the equation: \log_{10} survivors = $-0.0167 - 7.2485 \times \text{kGy} + 0.0317 \times \text{temperature} - 0.2470 \times \text{kGy} \times \text{temperature}$. R-square = 0.9665. The effects of both dose and the interaction of irradiation temperature with dose were significant. The equation predicts that each 10 degree decrease in temperature will result in a 0.92 and a 1.66 \log_{10} increase in survival at a dose of 0.5 and 0.8 kGy, respectively.

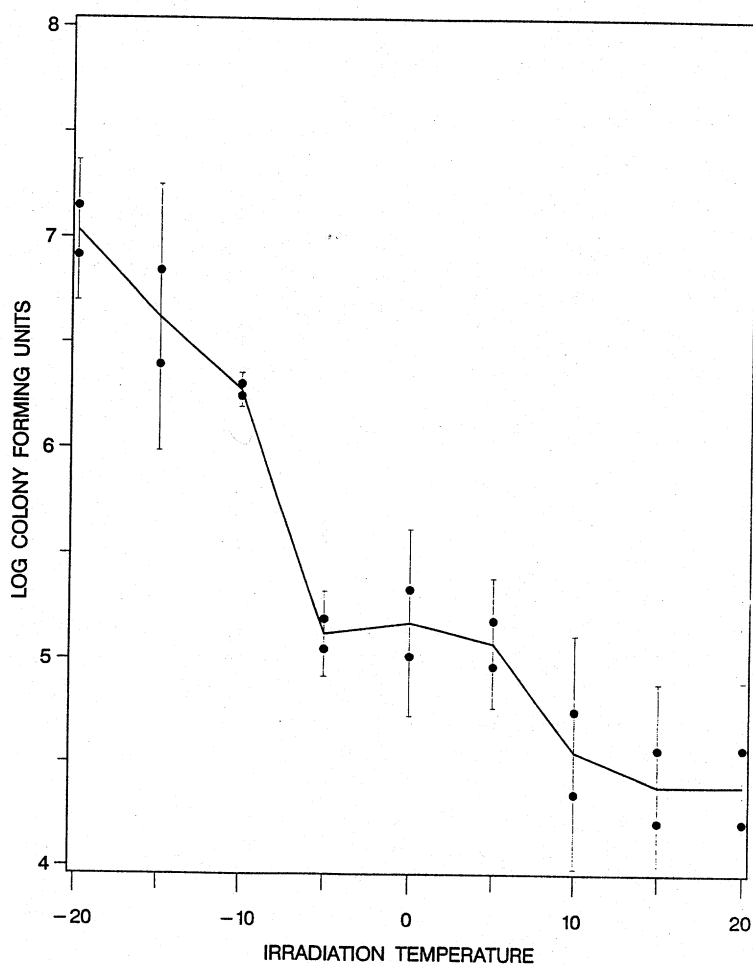


FIG. 1. SURVIVAL OF *S. putrefaciens* ON MECHANICALLY DEBONED CHICKEN MEAT FOLLOWING A RADIATION DOSE OF 0.5 kGy AT THE INDICATED TEMPERATURE, *IN VACUO*

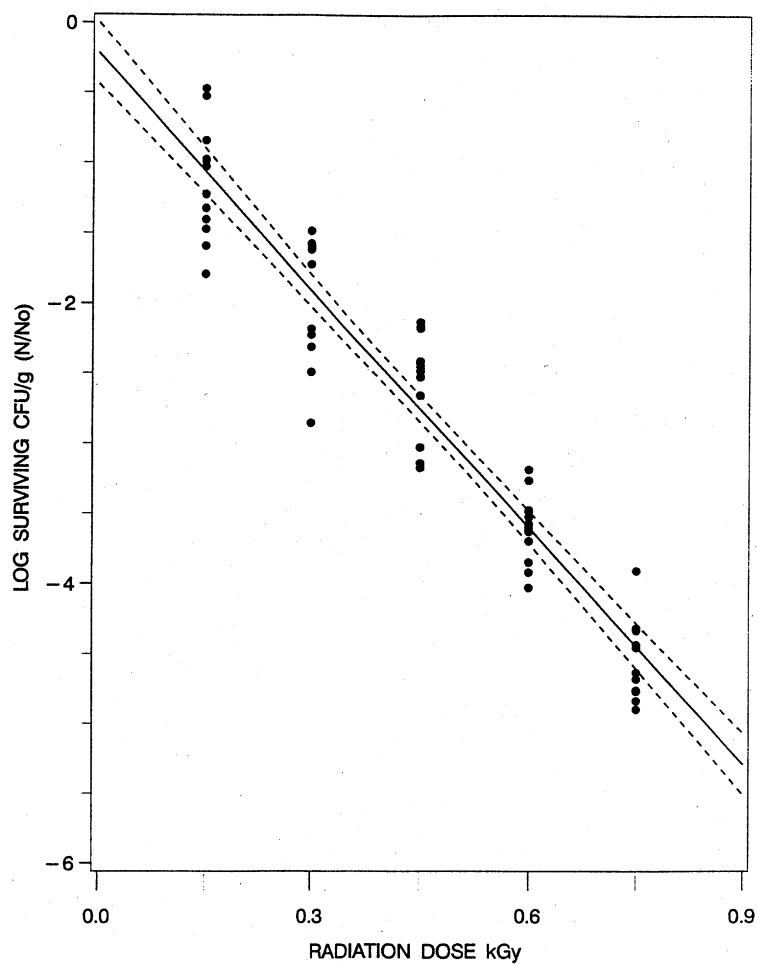


FIG. 2. SURVIVAL OF *S. putrefaciens* ON HAMBURGER, GROUND BEEF ROUND, GROUND PORK, AND GROUND TURKEY, *IN VACUO*, AT 5°C AND AT THE RADIATION DOSE INDICATED

The radiation D_{10} values for *S. putrefaciens* irradiated on hamburger, ground beef round, ground pork, and ground turkey were 0.19 ± 0.01 , 0.17 ± 0.01 , 0.17 ± 0.01 , and 0.18 ± 0.01 kGy, respectively. None of these values differed significantly from any one of the others. For this reason a single

regression was calculated from all 12 values for each dose (Fig. 2), and a D_{10} value was calculated from its slope. The combined D_{10} is 0.18 ± 0.01 kGy.

DISCUSSION

Bacterial contamination of fresh meat, poultry, and fish may occur during normal slaughter and handling procedures. Most of the contaminants are spoilage organisms, and some may be sensitive to the effects of gamma or electron irradiation. Reducing their numbers would increase the shelf-life of the products. There also might be fewer organisms that would produce typical spoilage odors, particularly under anaerobic conditions, should the product be temperature abused during storage.

Our results indicate that *S. putrefaciens*, with a D_{10} value of 0.18 kGy, would be unlikely to survive an absorbed ionizing radiation dose of 1.5 kGy, the current minimum dose allowed for the irradiation of poultry. A 12- D_{10} value would be approximately 2.16 kGy.

Though we would expect the presence of oxygen to decrease survival of irradiated *S. putrefaciens* no such effect was found. Perhaps the inherently large error that occurs in plate counts did not allow the difference in survival *in vacuo* to be distinguished from that in air. Even though the amount of meat was small, 5.0 g per sample, and its surface area was relatively large, its own metabolism may have sufficiently lowered the oxygen tension around the majority of bacteria to obscure any effects of oxygen. Similar effects have been noted with other bacteria irradiated under similar conditions (Thayer and Boyd 1992, 1994).

Impedance measurements (Fig. 2) indicate that ca. 70% of the cells surviving a dose of 0.7 kGy would be injured. This may indicate a reduced likelihood of their surviving another stress, such as refrigerated storage or heating during cooking.

Because the radiation resistance of *S. putrefaciens* is greater when it is irradiated at temperatures below freezing, some might survive a low radiation dose. However, its radiation resistance remains very low and it is unlikely, considering the low numbers present before irradiation, that any cells would survive a dose of 1.5 kGy. A gamma radiation dose of 1.5 kGy is equivalent to at least an 8D process for this bacterium.

When this organism was irradiated on different red meats or poultry under identical conditions, the radiation D_{10} values were not significantly different. All of these values were very low. This indicates that results obtained with one meat or poultry product can be extrapolated to include similar products under similar conditions of irradiation. The radiation resistance of *S. putrefaciens* is less than those of the foodborne pathogens *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* irradiated under similar conditions (Thayer *et al.* 1995b).

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